Metabolism of Acid Glycosaminoglycans in Granulation Tissue

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The age-dependent variation of the content of glycosaminoglycans in connective tissues has long been known, and differences have also been reported to exist [1–3] in the metabolism of the glycosaminoglycans in growing and mature animals. For investigations of this kind, implantation of viscose sponges [4,11] can be used to produce connective tissue at different stages of development. The nature and contents of glycosaminoglycans in experimental granulomas [5] of varying ages have recently been investigated. As an extension of that study, we followed the incorporation of labeled sulfate into glycosaminoglycans at stages where the positive net synthesis of collagen [4] and glycosaminoglycans [5] was rapid.

1 μCi/g of ^{35}S-sulfate (SJS. 1, The Radiochemical Centre, Amersham, England) was injected intraperitoneally into 14 to 15 weeks old female rats of the Wistar strain bearing 7-day or 25-day granulomas. The animals were killed after incorporation periods varying from 2 to 72 h. The granulomas were freed of extraneous tissue in a cold room and stored frozen. The glycosaminoglycans were liberated by papain digestion [6] (the undigested residues were discarded), the nucleic acids and proteins removed by precipitation with trichloroacetic acid (final concentration 5 %, w/v) and the glycosaminoglycans precipitated at 4° with a fourfold volume of ethanol saturated with sodium acetate. The precipitates were washed and dissolved in water and aliquots were analyzed for hexosamine [7] and labeled sulfate.

The radioactivities were measured with a Packard Tri-Carb Model 3214 scintillation spectrometer. The solvent was composed of methyl cellosolve (3 parts) and a "phosphor" solution (5 parts) containing 15 g of 2,5-diphenyloxazole (PPO) and 50 mg of 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) in one liter of toluene. The counting was continued until the statistical error was less than 1 % and the results were corrected for radioactive decay by reference to standard tables and for quenching by recounting all samples after the addition of an internal standard.

The samples were then lyophilized and redissolved in water to contain 1 μg of hexosamine per μl. Electrophoretic separation of the glycosaminoglycans was performed on cellulose acetate strips.[8] The strips were stained in a 1 % aq. solution of Alcian Blue and washed first with water and then with 5 % acetic acid to improve the contrast between the bands. The method used by Lehtonen [5,9] to identify electrophoretic fractions of glycosaminoglycans from experimental granulomas was followed. The spots containing the glycosaminoglycan fractions were cut from the strips (Fig. 1),

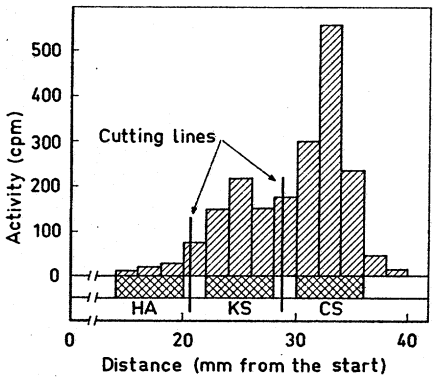

Fig. 1. Distribution of labeled sulfate in electrophoretic fractions of glycosaminoglycans from 25-day granuloma. After staining, the sheet was cut into 2-mm strips and each strip was counted separately. CS, chondroitin sulfate; KS, keratosulfate-resembling polysaccharide;[5] HA, hyaluronic acid. The keratosulfate-resembling polysaccharide contained glucosamine and galactose but no glucuronic acid, migrated in electrophoresis like keratosulphate and incorporated labeled sulfate.

dried at 80° and put into counting vials each containing 5 ml of the "phosphor" solution. The vials were allowed to stand for one hour at room temperature and cooled to 4° before counting (10 min). Some fractions were also dissolved from the strips and the radioactivities of their solutions were measured.[10] The efficiency of measuring of the radioactivities of the stained fractions on the cellulose acetate was found to be about 60–70 %. The ratios of the activities of the electrophoretic fractions were used to calculate

the activities of individual glycosaminoglycans from the specific activities (cpm/μg of hexosamine) of the isolated materials.

The Briggsian logarithms of the activities of the individual glycosaminoglycan fractions were plotted against the incorporation periods and the half-lives were estimated (Fig. 2). The half-lives of

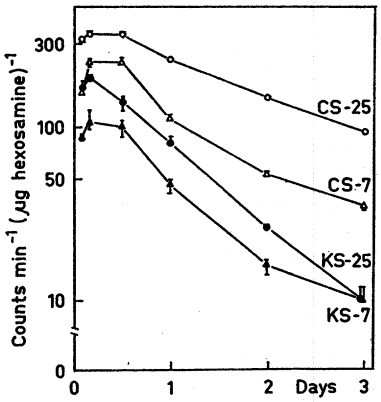

Fig. 2. Incorporation of ^{35}S-sulfate into glycosaminoglycans of 7-day and 25-day granulation tissues. Each sample was pooled from eight granulomas from two rats. The ranges are those from 3—6 electrophoretic separations.

chondroitin sulfates are 0.7—1.2 days in 7-day granuloma and 1.3—1.5 days in 25-day granuloma. The keratosulfate-resembling polysaccharide (see Fig. 1) has a half-life of 0.6—0.9 day in a 7-day granuloma and a half-life of 0.6—0.7 day in a 25-day granuloma. Because the separation of the radioactive fractions was not complete, the numerical values must be considered with some reserve.

No marked differences are observed between the 7-day and 25-day granulomas. The capacity of granulation tissue to synthesize collagen *in vitro* is maximal about 20 days after the implantation.[11] The nonlinearity of the incorporation curves for the 7-day granuloma suggests the existence of several metabolic compartments.

The results indicate a rapid turnover of glycosaminoglycans in granulation tissue. The half-lives are shorter in this tissue than in other tissues of rat and rabbit,[1–8,12–15] but also in carrageenin granulomas the neutral salt-soluble glycosaminoglycans have been shown to have a fast metabolism.[16] The rapid turnover of the keratosulfate-resembling polysaccharide is surprising, and further characterization of this fraction is needed. The keratosulfate of nucleus pulposus has been found to be metabolically inert and in this respect similar to fibrous collagen.[2]

Because the incorporation periods used in the present study were short, the half-lives may have been strongly affected by those glycosaminoglycans which are metabolized most rapidly. Further studies are necessary to clarify the relationships between collagen and glycosaminoglycans.

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